Ecological influences and morphological correlates of resting and maximal metabolic rates across teleost fish species

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ABSTRACT

Rates of aerobic metabolism vary considerably across evolutionary lineages, but little is known about the proximate and ultimate factors that generate and maintain this variability. Using data for 131 teleost fish species, we performed a large-scale phylogenetic comparative analysis of how interspecific variation in resting and maximum metabolic rates (RMR and MMR, respectively) is related to several ecological and morphological variables. Mass- and temperature-adjusted RMR and MMR are highly correlated along a continuum spanning a 30- to 40-fold range. Phylogenetic generalized least squares models suggest RMR and MMR are higher in pelagic species and that species with higher trophic levels exhibit elevated MMR. This variation is mirrored at various levels of structural organization: gill surface area, muscle protein content, and caudal fin aspect ratio (a proxy for activity) are positively related with aerobic capacity. Muscle protein content and caudal fin aspect ratio are also positively correlated with RMR. Hypoxia-tolerant lineages fall at the lower end of the metabolic continuum. Different ecological lifestyles are associated with contrasting levels of aerobic capacity, possibly reflecting the interplay between selection for increased locomotor performance on one hand and tolerance to low resource availability, particularly oxygen, on the other. These results support the aerobic capacity model of the evolution of endothermy, suggesting elevated body temperatures evolved as correlated responses to selection for high activity levels.

INTRODUCTION

Rates of energy expenditure vary several-fold among species, even after correction for factors such as body size and temperature (Hayes and Garland 1995; White and Kearney 2013; White and Seymour 2004). The proximate and ultimate causes of this variation and its ecological consequences remain active areas of research in ecological and evolutionary

physiology. The lower and upper limits to energy expenditure, corresponding to resting and maximum metabolic rates (RMR and MMR, respectively), affect several fitness-related traits, such as maintenance- and activity-related energy requirements, locomotor capacity and home-range area (Burton et al. 2011; Claireaux and Lefrancois 2007; Watanabe et al. 2015), and are consequently expected to be under multiple and often antagonistic selective pressures. Although a low RMR results in reduced maintenance energy requirements, a high MMR may increase foraging rates, behavioural dominance and overall competitive ability (Killen et al. 2014; Metcalfe et al. 1995; Watanabe et al. 2015). If so, this suggests that, in the absence of constraints, organisms should maximize their aerobic scope (i.e., the capacity to raise their level of aerobic metabolism, quantified as the difference between RMR and MMR). However, it has been hypothesized that the physiological machinery necessary to support a high aerobic capacity is metabolically costly (Burton et al. 2011; Killen et al. 2010; White and Seymour 2004), thus suggesting that RMR and MMR are not entirely independent from one another and may evolve in a correlated fashion in response to various ecological challenges.

Importantly, a mechanistic link between RMR and MMR may not only constrain the suite of possible responses to selection, but also give rise to evolutionary scenarios that could not be foreseen in the absence of this association. For example, according to the aerobic capacity model, endothermy in birds and mammals evolved as a by-product of selection for high locomotor performance and activity levels (Bennett 1991; Bennett and Ruben 1979). Although intra- and inter-specific associations between RMR and MMR have been studied extensively in endothermic lineages (Dutenhoffer and Swanson 1996; Hinds and Rice-Warner 1992; Koteja 1991; Rezende et al. 2004; Rezende et al. 2002; Sadowska et al. 2005; Wone et al. 2009), much less is known about how these metabolic rates are related in ectothermic species (but Taigen 1983, Walton 1993, Thompson and Withers 1997). More

extensive analyses are needed to determine the generality of relationships between RMR and MMR and their morphological, physiological, behavioural and ecological correlates across diverse ectothermic groups. For example, in environments where the benefits of an active lifestyle outweigh its energetic costs, selection for increased locomotor performance might favour the evolution of costly morphological and physiological traits at the expense of increased RMR, as originally proposed for the lineages giving rise to endothermic birds and mammals (Bennett and Ruben 1979). Understanding the mechanistic basis of variation in RMR and MMR is also crucial for assessing how selection on these traits affects lower levels of biological organization (e.g., tissues and organs), which might in turn pave the way for the origin of new ecological niches and evolutionary innovations. For example, recent evidence that the Root effect enhances oxygen delivery to the muscles (Rummer et al. 2013) suggests that the evolution of the swim bladder of ray-finned fishes may have resulted partly from selection for high aerobic capacity (Rezende 2013).

Here we examine how ecological demands on activity levels appear to affect aerobic performance across teleost fish species, and how these responses are related to variation in various morphological traits of likely importance. Teleosts present a unique opportunity to examine these questions because they constitute the most diverse taxonomic group of vertebrates, represent an enormous range of body sizes, and occupy a wide array of ecological niches with varying modes and levels of activity, with some highly active species even displaying varying degrees of endothermy (Block and Stevens 2001; Wegner et al. 2015). Our first major goal was to employ phylogenetically-informed interspecific analyses to investigate whether RMR, MMR, and aerobic scope are correlated across species. Absolute aerobic scope (AS = MMR - RMR) theoretically sets the capacity for simultaneous oxygen-consuming processes above maintenance levels, including activity, digestion and assimilation of nutrients, and coping with stressors or disease (Claireaux and Lefrancois

2007; Clark et al. 2013b; Fry 1971), whereas factorial aerobic scope (FAS = MMR/RMR) provides a relative measure of the maximum capacity for energy turnover with respect to resting expenditure rates, and is assumed to be relatively constant according to the aerobic capacity model (Rubenstein 2012). Because AS and FAS have different biological interpretations and may be associated with different ecological pressures (Clark et al. 2013b), we analysed how both of these measures of aerobic scope covary with RMR and MMR. Our second major goal was to test for associations between whole-organismal performance and relevant ecological variables on the one hand, and various key morphological traits on the other to identify, respectively, possible ultimate and proximate causes of the variation in metabolic rates that we observed.

METHODS

Metabolic Rates

Data on RMR and MMR, estimated from rates of oxygen consumption ($\dot{M}_{O_2}$), were collected from the literature for 92 species of teleost fishes. Data are deposited in the Dryad Digital Repository: DOI (Killen et al. 2016), as well as Table S1. Only species for which phylogenies are available were used in this study. When examining relationships between RMR and MMR, only data in which both variables were measured in the same study were used. There were, however, an additional 39 species for which RMR values alone were available, which were used to examine correlations between RMR and morphological traits. Only one dataset per species was used, to avoid giving undue weight to species represented by multiple studies (Killen et al. 2010). In such cases, priority was given to measures performed within the natural temperature range of a species, but closest to 15°C (to minimise the range of temperatures included in the dataset). A single averaged value was used for each

species. For MMR, measurements were only included from studies that made an effort to engage fish in maximal rates of active aerobic metabolism, measured either: 1) during peak levels of forced swimming (typically in a swim flume); 2) immediately following exhaustive exercise in a swim flume; or 3) immediately following exhaustive exercise by manual chasing. For RMR, we only accepted measurements from studies on fasted post-larval animals that included an attempt to eliminate oxygen uptake due to activity. In general this was performed by either: 1) extrapolating values of oxygen uptake ($\dot{M}_{O_2}$) measured during varying levels of activity (e.g. various swim speeds in a flume) to zero activity; or 2) measuring $\dot{M}_{O_2}$ during a period of quiet rest, during which $\dot{M}_{O_2}$ had stabilised after a period of acclimation to the respirometry chamber. In this regard, measures of RMR used in this study are likely to approximate to standard metabolic rate, which is the minimal level of aerobic metabolism required to sustain non-torpid life in ectotherms (Burton et al. 2011).

Ecological Variables

To examine how ecological demands on activity may relate to RMR and MMR, we obtained data on lifestyle and trophic level from Fishbase (www.fishbase.org) (Froese and Pauly 2008). We use 'lifestyle' as an integrative term to refer to aspects of phenotype (including life history, behaviour, morphology etc.), that tend to be associated with a particular niche, including living in a particular habitat type (Fuiman 1997; Hagen et al. 2000). Here, lifestyle of each species was classified as either 'pelagic', 'benthopelagic' or 'benthic'. Pelagic species are those that live in the open water column and generally feed near the water surface; benthopelagic species live and feed near (but not on) the substrate, sometimes associating with mid-waters or even surface waters depending on depth; and benthic species live on the bottom in direct contact with the substrate. Trophic level (TL)

reflects the position of a fish species within its food web (Mancinelli et al. 2013). To calculate TL, primary producers and detritus were assigned a TL of 1.0, with the TL of all additional fish species estimated as: TL = 1 + mean trophic level of prey (weighted by abundance). Smaller values reflect lower positions in the food web (i.e., herbivores and detritivores), whereas higher values reflect more piscivorous species.

Morphological Traits

To examine the effects of organ size and muscle composition on RMR and MMR, we collected data from the existing literature on liver and brain size, gill surface area, skeletal muscle protein content and caudal aspect ratio. These variables were selected for analysis because data were available in sufficient quantity for the same species for which we had data for RMR and MMR. Hepatosomatic index (HSI = liver mass / body mass) data were available from the literature for 27 of the species for which either RMR or MMR measurements (or both) existed. HSI is a standard measure in the fish literature and was used here because many studies did not provide either the mean raw liver mass or body mass independently. For each species the mean value from a particular study was used. Again, only one dataset per species was used, and when multiple datasets for the same species were available, we gave priority to the data collected closest to 15°C. For several studies (10 of 27 species) temperature was not reported in the original study (usually for data collected during field surveys). In these cases we assumed that the temperature was the same as that used to collect RMR and MMR, which was chosen because they were within the species' natural range.

Data for brain mass (% of body mass), gill surface area (cm^2), muscle protein content and caudal aspect ratio were obtained from Fishbase (www.fishbase.org; Ridet and Bauchot

1990, Froese and Pauly 2008). Muscle protein content was calculated as a percentage of fat- and ash-free muscle mass to minimize possible effects of differences in nutrition on biochemical composition. For brain mass, gill surface area and muscle protein content, multiple values from the same species were averaged using geometric means. When ranges were given, we used the midpoint. When species values were not available for brain mass and muscle composition, congeneric species values were used. Caudal fin aspect ratio describes the shape of the tail, which is used to propel fish while swimming, and is a correlate of average activity level across fish species (Pauly 1989). It is calculated as:

$A = h^2 / s$,

where A = aspect ratio; h = height of the caudal fin; and s = surface area of the caudal fin.

Because morphological traits were obtained from a different set of individuals than those used for metabolic data, the effects of body mass and temperature on these traits on the one hand, and on RMR and MMR on the other, could not be controlled statistically in a single multivariate model (see below). Consequently, we first inspected whether HSI, gill surface area, brain mass and muscle protein content were affected by body mass and temperature and removed these effects employing regression residuals when necessary (or adjusted estimates, which essentially correspond to residuals mapped onto the original dimensions by setting some covariates fixed; i.e., body mass = 1 kg and T_a = 15 ºC). Diagnostics showed that HSI was negatively correlated with ambient temperature, T_a (HSI = 2.88 – 0.069 T_a (ºC), $r^2 = 0.237$) and that gill surface area (gill SA) was positively related with body mass, M_b ($\log_{10}$ GA (cm^2) = 0.229 + 1.139 $\log_{10} M_b$ (g), $r^2 = 0.82$). Therefore, residuals of HSI regressed against temperature and log gill SA against log body mass were used in subsequent analyses. Brain mass was expressed as % body mass and was not related to body mass.

Statistical Analysis

To incorporate phylogenetic information into the analysis, we employed the dated phylogenies described by (Near et al. 2012) for Acanthomorpha (spiny-rayed fishes) and Wang et al. (2012) for Cypriniformes (carps) as the mainstays of our phylogenetic tree (Fig. 1). The relationship between these two and other basal groups was resolved following Zou et al. (2012). We then included information from additional sources to improve the resolution for specific monophyletic families: Anguillidae (eels) (Teng et al. 2009), Gadidae (cod) (Teletchea et al. 2006), Mugilidae (mullet) (Durand et al. 2012), Nototheniidae (cod icefish) (Kuhn and Near 2009), Pleuronectidae (flounder) (Cooper and Chapleau 1998), and Salmonidae (salmon) (Stearley and Smith 1993). Unresolved nodes were maintained as soft polytomies and the node depth in these cases was arbitrarily set to 1/2 the maximum depth.

All statistical analyses were performed with R (R Development Core Team, 2011). Statistical models incorporating phylogenetic information were generated using phylogenetic generalised least squares (PGLS) (Garland and Ives 2000) in the APE package (Paradis et al. 2004). RMR and MMR were always adjusted for body mass and water temperature effects employing the following standard PGLS model:

$$\log_{10} \mathrm{MR} = a_0 + a_1 \log_{10} M_\mathrm{b} + a_2 T_\mathrm{a} + \varepsilon$$

and calculating the adjusted estimates for M_b = 1 kg, T_a = 15°C and the residual variation ε. Although the effects of ecological and physiological correlates on MR were assessed with PGLS including additional variables to the standard model to minimize potential biases arising from correlating residuals from separate analyses (Freckleton 2009), adjusted values were employed to test for correlations between metabolic parameters morphological traits

because these variables were measured in different sets of individuals (and therefore the effects of covariates such as body mass and temperature had to be statistically removed in separate analyses).

For each PGLS model, a measure of phylogenetic correlation, λ (a multiplier of the off-diagonal elements of the covariance matrix, i.e. those quantifying the degree of relatedness between species) (Freckleton et al. 2002; Pagel 1999) was estimated by fitting models with different values of λ and finding the value that maximises the log likelihood. The value of λ can be used as a metric of the degree of phylogenetic correlation between traits (Freckleton et al. 2002). A maximum likelihood value of λ equal to 1 indicates a strong phylogenetic signal and demonstrates that the pattern of phenotypic covariation between the residuals of PGLS is best described by the original phylogeny, whereas a value of 0 indicates that the species data can be considered statistically independent and patterns of covariation resemble a star phylogeny. Intermediate values of λ specify models in which trait evolution is phylogenetically correlated, but to a lesser extent than expected under evolution by Brownian motion. The fit of different models was compared employing Akaike information criteria (AIC) (Freckleton 2009; Rezende and Diniz-Filho 2012) and the adequacy of each model with respect to alternative models was quantified with Akaike weights (w_i).

Data were log-transformed when necessary (i.e., for body mass, metabolism and subordinate traits that are expected to vary allometrically) and residuals were inspected to ensure that they were normally distributed. Models were tested after removing observations with a Cook's D > 0.5 to test whether results were affected by these influential points. For all analyses including muscle protein content, *Cyclopterus lumpus* was removed from the dataset because it had a value that was 4.35 standard deviations lower than the overall mean and thus had a large influence on model outputs (Cook's D = 3.4 and 3.6 for PGLS against adjusted RMR and MMR, respectively). To support interpretation of these analyses, null hypothesis

significance testing was employed to provide some indication of the strength of evidence for observed patterns, along with r^2. P-values are typically imprecise and arbitrary cut-offs for declaring statistical significance and are problematic and limiting in several ways (Boos and Stefanski 2011; Halsey et al. 2015). Thus, in the present article, the P-value is treated as a continuous variable providing an approximate level of evidence against the null hypothesis (Fisher 1959).

RESULTS

Allometry and ecological correlates

RMR and MMR were strongly associated with both body mass and temperature ($P < 0.004$ in all cases) following the relations (Fig. A1):

$$\log_{10} \mathrm{RMR} = -1.385 + 0.948 \log_{10} M_b + 0.021\ T_a \qquad (\lambda = 0.49, n = 112)$$

$$\log_{10} \mathrm{MMR} = -0.575 + 0.937 \log_{10} M_b + 0.015\ T_a \qquad (\lambda = 0.62, n = 79)$$

where metabolism is expressed in mg O_2 h^{-1}, M_b in g and T_a in ºC (95% CI for the allometric exponents are 0.882 – 1.014 for RMR and 0.864 – 1.011 for MMR). The coefficients obtained for thermal effects give rise to $Q_{10} = 1.62$ and 1.41 for RMR and MMR, respectively, which roughly correspond to a 4-fold increase in metabolic rates within the range of temperatures in which measurements were performed (between -1.5 and 30 ºC; Fig. A1). After controlling for these temperature effects, allometric exponents (± SE) for both RMR and MMR fall above the general expectation of 0.67 to 0.75 ($a_1 = 0.948 \pm 0.033$ and 0.937 ± 0.037, respectively). Adjusted RMR and MMR showed a 38- and 29-fold interspecific difference between minimum and maximum values, respectively (RMR: 9.25 – 347.67 mg O_2 h^{-1}; MMR: 59.93 – 1724.8 mg O_2 h^{-1}, adjusted to 1 kg and 15ºC), which

highlights the enormous range of variation in both RMR and MMR across species even after statistically removing temperature and body-mass effects (Fig. A2). Both measures of metabolic rate were positively skewed (RMR: skewness = 1.54±0.27, kurtosis = 2.90±0.45; MMR: skewness = 2.47±0.27, kurtosis = 8.21±0.54). After excluding the right tail of the distributions, the ranges in RMR and MMR still represent a 24-fold and 14-fold difference, respectively. Importantly, RMR and MMR were highly positively correlated, following the general relation $\log_{10}$ MMR = 1.22 + 0.68 $\log_{10}$ RMR, after adjusting for body mass and temperature effects (Phylogenetic Pearson's product-moment correlation, r = 0.721, P < 0.001; Fig. 2A).

We then compared different PGLS models to assess if ecology could account for any of the variation in RMR and MMR, including that for those species for which lifestyle and trophic level were known (Table 1). Estimates of λ indicate that mass- and temperature-adjusted RMR and MMR exhibit phylogenetic signal (i.e., closely related species tend to resemble each other; see Fig. 1), while the AIC and Akaike weights (w_i) suggest that inclusion of ecological variables, in particular lifestyle, improve model fit for both RMR and MMR. However, this improvement is more pronounced for MMR, since the standard model without ecological correlates had negligible support (AIC = 13.47, w_i = 0.01) while the inclusion of both lifestyle and trophic level resulted in the model with the best fit (AIC = 5.49, w_i = 0.60). The regression coefficient for trophic level in this model (0.091 ± 0.053) suggests a 2.1-fold increase in MMR as species climb from the bottom to the top of the food web based on trophic level estimates (ranges from 1 to 4.5), everything else being equal. The effect of lifestyle was consistent across metabolic variables (RMR and MMR) and PGLS models, suggesting that pelagic species exhibit higher metabolic rates than do their benthic and benthopelagic counterparts (P < 0.02 in all models; Fig. 2A). The higher MMR of pelagic species partly accounts for the positive association between adjusted MMR and RMR (Fig.

2), as evidenced by a shallower slope when lifestyle is included in the model ($\log_{10}$ MMR = 1.45 + 0.62 $\log_{10}$ RMR in this case) and a substantially better fit (AIC = – 58.6 versus – 51.6). However, RMR and MMR remain highly correlated ($P < 0.001$) after adjusting for the effect of lifestyle, and therefore additional factors must influence this association.

Aerobic scope

There was a 30-fold difference in AS across teleost species in our dataset (AS: 50.7 – 1504.5 mg O_2 h^{-1}; Fig. 2B and C) and a 6.9-fold range of variation in FAS (FAS: 1.80 – 12.36 × RMR; Fig. 2D). The relationship between AS and RMR was strong (Phylogenetic Pearson's product-moment correlation, $r = 0.512$, $P < 0.001$) and between AS and MMR was very strong ($r = 0.960$, $P < 0.001$; Fig. 2C). Consideration of these relationships suggests that variation in AS is driven primarily by variation in MMR, and that the impact of elevated RMR on variation in AS is essentially negligible at the interspecific level. Not only were the correlations between AS and MMR very close to 1 and the range of variation in AS and MMR nearly identical, but also the association between AS and RMR is expected to be negative (AS = MMR – RMR) unless species with an elevated RMR also exhibit at least an equal elevation in MMR.

In contrast, variation in FAS showed a very weak association with MMR ($r = 0.101$, $P = 0.38$; Fig. 2D), and therefore the energy costs of having an elevated MMR translate into a high RMR in both absolute and relative terms. For instance, the relationship between metabolic estimates adjusted for body mass and temperature (see above) shows that a 5-fold increase in MMR results in a nearly 11-fold rise in RMR. In summary, our analyses (Table 1, and Fig. 2) suggest that ecological differences across teleost fishes partly account for the variation in RMR and MMR, and that a high AS has likely been favoured by selection in

species with an active lifestyle despite a correlated rise in baseline energy costs accompanying investment in a higher MMR. Accordingly, comparisons between PGLS models for AS and FAS also support the inclusion of ecological variables in the model (the pooled Akaike weight supporting ecological models was, respectively, w_i = 0.99 and 0.71; Table A1).

Morphological traits

The results from analyses of relationships between adjusted RMR and MMR and morphological traits are presented in Table 2. Only muscle protein content and caudal fin aspect ratio were positively correlated with both metabolic rates, and in both cases the association was stronger for MMR (both the slope estimates and r^2 were consistently higher for MMR; Table 2). Based on the range of these variables in our dataset and slope estimates of PGLS (Table 2), variation in muscle protein content between species (range: 15.7 – 24.2%) translates into 2.4-fold and 5.1-fold differences in RMR and MMR, whereas variation in caudal fin aspect ratio (range: 0.66 – 7.2) is associated with 3.4-fold and 4.4-fold differences in RMR and MMR, respectively (Fig. 3). Gill surface area was not correlated with RMR, whereas its relationship with MMR was highly affected by *Sander lucioperca* (Cook's D = 0.621). Although the correlation was weak when this species was included in analyses, removal of this influential data point results in a positive correlation between these variables (Table 2; Figure 3C) and an estimated 3.1-fold difference in MMR as a function of variation in gill surface area (range: 1,005 – 18,924 cm^2 adjusted for a 1 kg fish). There was also some evidence of an association between liver size and RMR (varying 2-fold in relation to an HSI range of 0.25-5.90), but not MMR (Table 2). There was no evidence that RMR or MMR are related to brain mass (Table 2).

We could not include all morphological traits in the same model given the fragmented nature of the dataset (e.g., a PGLS including MMR, muscle protein content, gill surface area and aspect ratio is reduced to n = 16 spp.) and the high degree of collinearity between some morphological traits (Fig. 3). According to Pearson's product-moment correlation analyses, muscle protein content and caudal fin aspect ratio were highly correlated ($r_{23} = 0.89$ and $P < 0.001$), and the same is true for the association between these variables and gill surface area ($r_{25} = 0.42$, $P = 0.03$ and $r_{18} = 0.53$, $P = 0.016$, respectively). Importantly, differences across species in these traits appear to be primarily affected by the high muscle protein content, caudal fin aspect ratio and gill surface area of pelagic species (Fig. 3), as suggested by the PGLS including lifestyle as a factor ($F_{2,50} = 8.8$, $P < 0.01$ for muscle protein content; $F_{2,29} = 8.4$, $P = 0.0013$ for gill surface area and $F_{2,26} = 4.18$, $P = 0.027$ for caudal fin aspect ratio). Overall, these results suggest that more active species have evolved higher metabolic rates and other supporting morphological adaptations at the organ and tissue levels.

DISCUSSION

Our results show that teleost fish exhibit enormous interspecific variation in both RMR and MMR after standardising for body mass and temperature, far surpassing the variation previously documented for endotherms (Rezende et al. 2004; Rezende et al. 2002; White and Seymour 2004). Furthermore, we have found that RMR and MMR are highly positively correlated, which can be depicted as a broadly bounded metabolic continuum (see Fig. 2A). We hypothesize that this extensive, coordinated variation in RMR and MMR has resulted from the opposing effects of conflicting ecological demands. At the high end of the continuum, selection for increased locomotor performance in species with pelagic and high trophic level lifestyles appears to favour high MMR, RMR and AS, whereas at the low end of

the continuum, selection for tolerance to low or patchy resource availability may favour reduced RMR, MMR and AS. Our study has also revealed that the variation of three very different morphological traits (gill surface area, muscle protein content and caudal fin aspect ratio) is associated with this metabolic continuum, thus providing an extraordinary example of the centrality of organismal performance in integrative and evolutionary biology (Arnold 1983; Garland and Carter 1994; Kingsolver and Huey 2003). In short, we suggest that opposing selection for increased behavioural activity versus a greater tolerance of oxygen or food limitation has resulted in the coordinated evolution of resting and maximal metabolic rates, as well as diverse morphological traits supporting these rates. Each of these proposed types of selection is discussed further below.

Selection for Increased Locomotor Capacity

According to our hypothesis, the adoption of an active pelagic lifestyle has favoured fish with an increased capacity for locomotor performance, which is functionally supported by a suite of morphological and physiological responses at lower levels of organization. A comparison among alternative PGSL models (Table 1) suggests that ecology is crucial in explaining the interspecific variation in MMR, with models including ecological variables exhibiting an overall support of 99% based on the sum of Akaike weights (w_i). For RMR, the overall support of 70% for ecological models was more modest in spite of the larger sample size. Additionally, the strength of the correlation between metabolic and morphological traits, quantified as either the slope estimates of the PGSL model or the r^2 (Table 2), was also consistently higher for MMR than RMR (Fig. 3). The stronger effect of activity-related, ecological life style on MMR than RMR suggests that selection on increased locomotor capacity may have acted primarily on MMR with a secondary effect on RMR, a hypothesis requiring further testing (also see next paragraph). Variation in AS across species was predominantly explained by variation in MMR; theoretically a larger AS provides valuable

capacity to perform aerobic exercise, recover from anaerobic exercise, and also deal with contingencies such as disease or other environmental stressors (Fry 1971, Claireaux and Lefrancois 2007). As a result, selection should favour an increased AS or MMR, all other factors being equal. For example, it has been argued that a main benefit of an increased MMR in scombroids is a larger AS and reduced constraints on digestion, gonadal growth, and recovery from intense anaerobic exercise (Brill 1996).

Importantly, we found a strong positive correlation between RMR and MMR that extends across fish species of various lifestyles from sluggish benthic species to high-performance pelagic species, suggesting that an elevated aerobic capacity entails important energy costs. However, a higher RMR may also be favoured by natural selection if it helps support partial endothermy and higher activity levels and associated MMR (or higher growth rates; see (Burton et al. 2011). As with birds and mammals, increased RMR gives rise to elevated body temperatures which, in turn, enhances energy turnover rates and aerobic performance (Clarke and Pörtner 2010). Even though the cost of transport for swimming animals is low compared to those that walk or fly (Schmidt-Nielsen 1972), which probably explains why many fish species exhibit a broad range of aerobically supported locomotory speeds with a relatively low MMR (Bennett and Ruben 1979; Hillman et al. 2013), it appears that in temperate waters there is also strong selection on the capacity for remaining active.

A few fish species (e.g., some tunas, billfishes, sharks) engage in partial endothermy to maintain the temperature of certain organs at many degrees above the ambient water temperature (Block and Stevens 2001), which also results in increased activity and swimming performance (Watanabe et al. 2015). These cases of endothermy in highly active pelagic predators including the recently described endothermic fish *Lampis guttatus* (Wegner et al. 2015), in conjunction with our results showing that MMR and RMR are highly correlated, provide strong support for the aerobic capacity model in some fish lineages. According to the

aerobic capacity model (Bennett and Ruben 1979), a physiological linkage between RMR and MMR reflects a factorial limitation in oxygen-processing ability across vertebrate lineages that should also translate into a low FAS varying within a relatively narrow range. Our results support this prediction: the empirical range of variation in FAS (1.80 to 12.36) is considerably smaller than the expected variation in the absence of an association between RMR and MMR (ranging from ~1 to 186.4 × RMR, based on ratios calculated from randomized adjusted estimates of RMR and MMR). As a cautionary note, adjusting for water temperature effects on the metabolism of partially endothermic fishes can be complicated by activity levels and should be performed with care (Blank et al. 2007; Clark et al. 2013a).

Selection for Tolerance to Resource Limitation

For some environments or niches even a small increase in RMR, associated with an incremental increase in MMR, could constrain species from evolving a higher maximal aerobic capacity because of the increased energy requirements to maintain baseline metabolism. Furthermore, depending upon the ecological niche, species may gain little advantage from an elevated MMR or AS. For example, the results of the present study suggest that relatively inactive species, or those at lower trophic levels, possess lower MMRs, as compared to active species or those at higher trophic levels. As a result, adaptation to varying ecological demands among species appears to generate interspecific variation in RMR and MMR that largely follows a continuum between a high capacity for activity with high maintenance costs, and a low capacity for activity with low maintenance costs (Fig. 4).

Although there are several potential benefits associated with having a high aerobic capacity, some of the pressures favouring lower RMR may be inferred by studying those species at the opposite end of the metabolic continuum. The lowest levels of adjusted RMR were observed in cyprinids (17 of the 28 lowest records occurred within the Cyprinidae; Fig.

1), a highly diverse family with many species exhibiting very high tolerance levels to hypoxia and anoxia. Oxygen has a low solubility in water and obtaining access to sufficient oxygen can be a challenge for organisms living in many aquatic habitats (Diaz and Rosenberg 2008). When faced with hypoxic and hypercapnic conditions, several fish species increase oxygen extraction and transport efficiency by modulating gill surface area, oxygen affinity of haemoglobin and muscle mitochondrial density (Dhillon et al. 2013; Fu et al. 2014; Nilsson and Renshaw 2004). Additionally, at least three cyprinid species (*Carassius carassius*, *C. auratus* and *Rhodeus amarus*) are known to exhibit a specialised metabolic pathway that enables them to survive several months in anoxic cold waters (Nilsson and Renshaw 2004). In our dataset, species belonging to the genera *Carassius*, *Rhodeus*, *Cyprinus*, *Hypophthalmichthys* and *Aristichthys*, which are known to tolerate very high levels of hypoxia (Dhillon et al. 2013; Fu et al. 2014), exhibit very low levels of adjusted RMR (range: 14.6 – 25.5 mg O_2 h^{-1}). Moreover, the low adjusted RMR for *Typhlogobius californiensis* (Gobiidae), *Genyagnus monopterygius* (Uranoscopidae), *Anguilla rostrata* and *A. japonica* (Anguillidae) confirm that a reduced RMR is common in other hypoxia tolerant lineages. These results not only support recent experimental work suggesting that adaptation for high activity may trade-off with hypoxia tolerance in fishes (Crans et al. 2015; Fu et al. 2014; Stoffels 2015), but also suggest that this phenomenon may generate interspecific diversity in metabolic and performance traits across a broad range of teleost lineages.

Other benefits of a reduced RMR could include tolerance to food-deprivation and an ability to occupy habitats or niches with low or sporadic food availability. Within species, high RMR is associated with reduced growth rate under conditions of low food availability (Burton et al. 2011; Killen et al. 2011). Consequently, fishes that experience frequent hypoxia or patchy food availability due to their ecology (e.g., burrowers, some benthic sit-and-wait

predators and species subject to tidal exposure and/or eutrophication) are expected to evolve reduced RMR.

Modifications to Morphological Traits

The hypothesis that selection acts on aerobically-supported locomotor performance is compatible with results observed at lower levels of biological organization, i.e. relatively active pelagic species also exhibit more protein-rich skeletal musculature and increased gill surface area. Elevated muscle protein content may be related to an increased density of muscle fibres, myofibrils, and (or) mitochondria (Johnston et al. 1998), which should support increased aerobic ATP turnover during movement, and faster and more forceful rates of contraction (Altringham and Shadwick 2001), while also being more costly to maintain (Houlihan 1991). This twin association of muscle protein content with activity level and maintenance costs may partly account for the covariation between adjusted RMR and MMR that has been observed (Table 2, Fig. 3). Caudal fin aspect ratio is a direct correlate of activity levels in fish species (Pauly 1989), and it was positively correlated with RMR, MMR, and muscle protein content in the present study. It is therefore possible that ecological demands on physical activity have influenced associations between RMR and MMR via proximate effects of specific morphological and biochemical features, such as those that we have identified. It should be noted that in fish, aerobic "red" muscle used for sustained swimming and the mainly anaerobic "white" muscle used for burst-type swimming are arranged in discrete sections (Dickson 1996), and the majority of studies used in our analysis sampled the white, mainly anaerobic, musculature. However, although white muscle is not the main contributor to activity at sustainable swimming speeds, there can be a substantial anaerobic contribution to activity as an animal approaches this peak and so more active species should employ more white muscle protein to work in conjunction with red muscle fibres. Having a higher aerobic capacity can also help recovery from high levels of anaerobic exercise (Brill

1996; Killen et al. 2014). Finally, although predominantly anaerobic, white muscle also contains some mitochondria and large amounts of protein that need to be maintained while the animal is at rest, which is particularly true for high-energy-demand species like the tunas (Dickson 1996).

The observed association between gill surface area and MMR, but not RMR, supports previous reports that more active fish species have a greater gill-surface area (Schmidt-Nielsen 1984) and suggests that MMR may be constrained by the capacity to extract oxygen from the water. Along with muscle protein content, gill surface area is intrinsically related to oxygen extraction, transport and utilization, and is also highly correlated with caudal fin aspect ratio (Fig. 4). In aquatic animals, gills not only facilitate gas exchange and blood oxygenation, but also function in the processes of ion-regulation, acid-base balance, and nitrogen excretion, as evidenced by the numerous ion channels, pumps, and exchangers embedded within their epithelium (Marshall and Grossell 2006). In theory, the multiple functions of gills and associated machinery could increase maintenance costs of this tissue. However, a link between RMR and gill SA was not observed, perhaps because correlations between metabolic rates and gill SA may be obscured by a number of additional factors. Although selection for enhanced locomotor performance should increase gill SA, adaptation for hypoxia tolerance could also increase gill SA in more sluggish species. Furthermore, in cases where tolerance to resource-limitation is a predominant target of selection, different evolutionary outcomes may arise depending on which resource is limiting: gill SA could increase if oxygen supply is limiting, but probably not if food is scarce.

The evolution of metabolic rates via selection on capacity for activity or tolerance to resource limitation could limit the evolutionary trajectory of organ systems in ways that may not be immediately apparent or that obscure links between organismal metabolic rates and the sizes and functions of some organs. In the current study, for example, brain mass was not

related to either RMR or MMR, despite being an organ that is highly metabolically active (Rolfe and Brown 1997). For a given level of RMR, however, a species may evolve a large brain, but 'compensate' by having a smaller liver or gut; correspondingly, species with a larger liver or gut may evolve smaller brains (Tsuboi et al. 2015). This variation in the proportional contribution of specific organs to metabolic rates among species could in theory damp correlations between some morphological traits and metabolic rates and play an important role in how suites of organ-level traits evolve in response to ecological pressures on whole-animal performance. This could partially explain variable findings of previous studies examining correlations between metabolic rates and organ sizes across and within species (Hayes and Garland 1995; Norin and Malte 2012; Swanson et al. 2012; Zhang et al. 2014).

Due to the fragmented nature of the dataset, our analysis is correlational and so we must consider other potential explanations for the observed results. For example, it is conceivable that the strong relationship between RMR and MMR may have at least partially arisen through direct environmental effects, i.e. phenotypically plastic acclimation rather than genotypic adaptation. It is also possible that selection has acted directly on one or more of the morphological traits considered in this study (e.g. gill surface area), which then resulted in correlated selection on MMR. Additional data on morphological traits would be valuable to allow path analyses that could suggest the primary targets of selection and likely causal pathways. It is also interesting that although the slope of the correlation between RMR and MMR decreases when lifestyle is included in the PGLS model, a positive relationship remains, suggesting that other factors not considered here may also be playing a role. For example, differences in foraging mode within a trophic level or lifestyle – such as where a species lies on the continuum between sit-and-wait and active foraging (Lourdais et al. 2014) – may influence activity level and possibly the strength of correlated selection mong traits.

Comparison to Other Ectotherms

Our findings generally agree with previous work showing positive interspecific relationships among RMR and MMR (e.g. Daan et al. 1990; Raichlen et al. 2010; Rezende et al. 2004; White and Kearney 2013; Wiersma et al. 2007). However, most studies have focussed on endothermic taxa, whereas relatively little data on these relationships exists for ectotherms. Taigen (1983) reported a positive correlation between MMR and RMR among 17 species of anuran amphibians and suggests that MMR may be linked to predatory behavior in anurans. Walton (1993) found that hylid frogs have a similar positive correlation between MMR and RMR after correction for phylogeny, suggesting that temperate hylids have evolved a higher MMR and RMR than tropical hylids, possibly to allow high activity at colder temperatures. Gomes et al. (2004) found mixed intraspecific relationships between RMR and MMR in anurans, but observed that active species were more likely to show a positive correlation. Widely foraging goannas (*Varanus* spp.) tend to have a higher RMR as compared to more sedentary species (Thompson and Withers 1997). Andrews and Pough (1985) reported that RMR in squamate reptiles is also related to predatory lifestyle. For insects, flying species have more energetically costly locomotion and also have significantly higher RMR than non-flying species (Reinhold 1999). While these findings generally support our conclusions, additional large, phylogenetically informed analyses on other ectotherms besides fish are needed to test whether the patterns that we have observed are widely applicable.

Conclusions

Although correlational in nature, several lines of evidence suggest that ecological demands on behaviour/locomotor activity and tolerance to oxygen or food limitation are key targets of selection in teleost fishes that result in correlated responses in performance and

various supporting metabolic and morphological traits. For example, natural selection for increased activity appears to have been supported functionally by increasing the interrelated traits of MMR, RMR, gill SA, caudal fin aspect ratio, and muscle protein content. By contrast, selection for tolerance to resource limitation may be supported by decreasing MMR, RMR and muscle protein content, whereas gill SA may decrease or increase depending on whether oxygen or food is limiting. Additional work is needed to understand how selection on locomotor capacity and tolerance to resource limitation interact with other potential targets for natural selection.

Our analyses also show that mass- and temperature-adjusted RMR and MMR are highly positively correlated, which suggests that selection for increased maximal rates of aerobic metabolism generates elevated maintenance costs and, in some extreme cases, may lead to the evolution of endothermy. Teleost fishes may provide a highly relevant model to study the aerobic capacity model for the evolution of endothermy (Bennett and Ruben 1979), given mounting evidence that multiple lineages exhibit some degree of endothermy (Block and Stevens 2001; Wegner et al. 2015; Welsh and Bellwood 2012) and that elevated body temperatures translate into detectable ecological effects (Watanabe et al. 2015). More generally, our results highlight that, because of their enormous range of phenotypic variation, teleosts constitute an excellent group to investigate how varying lifestyles and evolutionary pressures can ultimately give rise to an astonishing diversity in form and function.

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APPENDICES

Figure A1: Mass-scaling and temperature effects on metabolic rates of teleosts.

Figure A2: Frequency distributions for resting metabolic rate (RMR; upper panel) and maximum metabolic rate (MMR; lower panel) standardised for mass (1 kg) and temperature (15°C), for all fish species included in analyses.

REFERENCES

Altringham, J. D., and R. E. Shadwick. 2001. 8. Swimming and muscle function, Pages 313-344 *in* B. Barbara, and E. Stevens, eds. Fish Physiology, Academic Press.

Arnold, S. J. 1983. Morphology, performance and fitness. American Zoologist 23:347-361.

Bennett, A. F. 1991. The evolution of activity capacity. Journal of Experimental Biology 160:1-23.

Bennett, A. F., and J. A. Ruben. 1979. Endothermy and activity in vertebrates. Science 206:649-654.

Blank, J. M., J. M. Morrissette, C. J. Farwell, M. Price, R. J. Schallert, and B. A. Block. 2007. Temperature effects on metabolic rate of juvenile Pacific bluefin tuna Thunnus orientalis. Journal of Experimental Biology 210:4251-4261.

Block, B. A., and E. D. Stevens. 2001, Tuna: physiology, ecology, and evolution, v. 19, Gulf Professional Publishing.

Boos, D. D., and L. A. Stefanski. 2011. P-Value precision and reproducibility. The American Statistician 65:213-221.

Brill, R. W. 1996. Selective advantages conferred by the high performance physiology of tunas, billfishes, and dolphin fish. Comparative Biochemistry and Physiology Part A: Physiology 113:3-15.

Burton, T., S. S. Killen, J. D. Armstrong, and N. B. Metcalfe. 2011. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proceedings of the Royal Society B: Biological Sciences 278:3465-3473.

Claireaux, G., and C. Lefrancois. 2007. Linking environmental variability and fish performance: integration through the concept of scope for activity. Philosophical Transactions of the Royal Society B: Biological Sciences 362:2031-2041.

Clark, T. D., C. J. Farwell, L. E. Rodriguez, W. T. Brandt, and B. A. Block. 2013a. Heart rate responses to temperature in free-swimming Pacific bluefin tuna (Thunnus orientalis). Journal of Experimental Biology 216:3208-3214.

Clark, T. D., E. Sandblom, and F. Jutfelt. 2013b. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. The Journal of Experimental Biology 216:2771-2782.

Clarke, A., and H.-O. Pörtner. 2010. Temperature, metabolic power and the evolution of endothermy. Biological Reviews 85:703-727.

Cooper, J., and F. Chapleau. 1998. Monophyly and interrelationships of the family Pleuronectidae (*Pleuronectiformes*), with a revised classification. Fishery Bulletin 96:686-726.

Crans, K. D., N. A. Pranckevicius, and G. R. Scott. 2015. Physiological tradeoffs may underlie the evolution of hypoxia tolerance and exercise performance in sunfish (Centrarchidae). Journal of Experimental Biology 218:3264-3275.
Daan, S., D. Masman, and A. Groenewold. 1990. Avian basal metabolic rates: their association with body composition and energy expenditure in nature. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 259:R333-R340.
Dhillon, R. S., L. Yao, V. Matey, B.-J. Chen, A.-J. Zhang, Z.-D. Cao, S.-J. Fu et al. 2013. Interspecific differences in hypoxia-induced gill remodeling in carp. Physiological and Biochemical Zoology 86:727-739.
Diaz, R. J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science 321:926-929.
Dickson, K. A. 1996. Locomotor muscle of high performance fishes - what do comparisons of tunas with ectothermic sister taxa reveal? . Comparative Biochemistry and Physiology 113:39-49.
Durand, J.-D., K.-N. Shen, W.-J. Chen, B. W. Jamandre, H. Blel, K. Diop, M. Nirchio et al. 2012. Systematics of the grey mullets (Teleostei: Mugiliformes: Mugilidae): molecular phylogenetic evidence challenges two centuries of morphology-based taxonomy. Molecular phylogenetics and evolution 64:73-92.
Dutenhoffer, M. S., and D. L. Swanson. 1996. Relationship of basal to summit metabolic rate in passerine birds and the aerobic capacity model for the evolution of endothermy. Physiological Zoology 69:1232-1254.
Fisher, R. 1959, Statistical Methods and Scientific Inference. New York, Hafner Publishing.
Freckleton, R. 2009. The seven deadly sins of comparative analysis. Journal of Evolutionary Biology 22:1367-1375.
Freckleton, R. P., P. H. Harvey, and M. Pagel. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. American Naturalist 160:712-726.
Froese, R., and D. Pauly. 2008. FishBase, World Wide Web electronic publication.
Fry, F. E. J. 1971. The Effect of Environmental Factors on the Physiology of Fish, Pages 1-98 Fish Physiology, Academic Press.
Fu, S.-J., C. Fu, G.-J. Yan, Z.-D. Cao, A.-J. Zhang, and X. Pang. 2014. Interspecific variation in hypoxia tolerance, swimming performance and plasticity in cyprinids that prefer different habitats. The Journal of Experimental Biology 217:590-597.
Fuiman, L. A. 1997. What can flatfish ontogenies tell us about pelagic and benthic lifestyles? Journal of Sea Research 37:257-267.
Garland, T., and P. Carter. 1994. Evolutionary physiology. Annual Review of Physiology 56:579-621.
Garland, T., Jr., and A. R. Ives. 2000. Using the past to predict the present: Confidence intervals for regression equations in phylogenetic comparative methods. American Naturalist 155:346-364.
Hagen, W., G. Kattner, and C. Friedrich. 2000. The lipid compositions of high-Antarctic notothenioid fish species with different life strategies. Polar Biology 23:785-791.
Halsey, L., D. Curran-Everett, S. Vowler, and G. Drummond. 2015. The fickle P value generates irreproducible results. Nature Methods 12:179-185.
Hayes, J. P., and T. Garland, Jr. 1995. The Evolution of Endothermy: Testing the Aerobic Capacity Model. Evolution 49:836-847.
Hillman, S. S., T. V. Hancock, and M. S. Hedrick. 2013. A comparative meta-analysis of maximal aerobic metabolism of vertebrates: implications for respiratory and cardiovascular limits to gas exchange. Journal of Comparative Physiology B 183:167-179.
Hinds, D. S., and C. N. Rice-Warner. 1992. Maximum metabolism and aerobic capacity in heteromyid and other rodents. Physiological Zoology 183:188-214.
Houlihan, D. F. 1991. Protein Turnover in Ectotherms and Its Relationships to Energetics, Pages 1-43 Advances in Comparative and Environmental Physiology. Advances in Comparative and Environmental Physiology, Springer Berlin Heidelberg.

Johnston, I. A., J. Calvo, H. Guderley, D. Fernandez, and L. Palmer. 1998. Latitudinal variation in the abundance and oxidative capacities of muscle mitochondria in perciform fishes. The Journal of Experimental Biology 201:1-12.
Killen, S. S., D. Atkinson, and D. S. Glazier. 2010. The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecology Letters 13:184-193.
Killen, S. S., D. Glazier, E. L. Rezende, T. Clark, D. Atkinson, A. Willener, and L. G. Halsey. 2016. Data from: Metabolic rates in teleost fishes: ecological determinants and physiological correlates.
Killen, S. S., S. Marras, and D. J. McKenzie. 2011. Fuel, fasting, fear: Routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. Journal of Animal Ecology 80:1024-1033.
Killen, S. S., M. D. Mitchell, J. L. Rummer, D. P. Chivers, M. C. O. Ferrari, M. G. Meekan, and M. I. McCormick. 2014. Aerobic scope predicts dominance during early life in a tropical damselfish. Functional Ecology 28:1367-1376.
Kingsolver, J. G., and R. B. Huey. 2003. Introduction: the evolution of morphology, performance, and fitness. Integrative and Comparative Biology 43:361-366.
Koteja, P. 1991. On the Relation Between Basal and Field Metabolic Rates in Birds and Mammals. Functional Ecology 5:56-64.
Kuhn, K. L., and T. J. Near. 2009. Phylogeny of Trematomus (Notothenioidei: Nototheniidae) inferred from mitochondrial and nuclear gene sequences. Antarctic Science 21:565-570.
Lourdais, O., G. E. Gartner, and F. Brischoux. 2014. Ambush or active life: foraging mode influences haematocrit levels in snakes. Biological Journal of the Linnean Society 111:636-645.
Mancinelli, G., S. Vizzini, A. Mazzola, S. Maci, and A. Basset. 2013. Cross-validation of δ15N and FishBase estimates of fish trophic position in a Mediterranean lagoon: The importance of the isotopic baseline. Estuarine, Coastal and Shelf Science 135:77-85.
Marshall, W. S., and M. Grossell. 2006, Ion transport, osmoregulation, and acid-base balance: The Physiology of Fishes. Boca Raton, CRC Press.
Metcalfe, N. B., A. C. Taylor, and J. E. Thorpe. 1995. Metabolic rate, social status and life-history strategies in Atlantic salmon. Animal Behaviour 49:431-436.
Near, T. J., R. I. Eytan, A. Dornburg, K. L. Kuhn, J. A. Moore, M. P. Davis, P. C. Wainwright et al. 2012. Resolution of ray-finned fish phylogeny and timing of diversification. Proceedings of the National Academy of Sciences 109:13698-13703.
Nilsson, G. E., and G. M. C. Renshaw. 2004. Hypoxic survival strategies in two fishes: extreme anoxia tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark. Journal of Experimental Biology 207:3131-3139.
Norin, T., and H. Malte. 2012. Intraspecific variation in aerobic metabolic rate of fish: relations with organ size and enzyme activity in brown trout. Physiological and Biochemical Zoology 85:645-656.
Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature 401:877-884.
Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics 20:289-290.
Pauly, D. 1989. Food consumption by tropical and temperate fish populations: some generalizations. Journal of Fish Biology 35:11-20.
Raichlen, D., A. Gordon, M. Muchlinski, and J. J. Snodgrass. 2010. Causes and significance of variation in mammalian basal metabolism. Journal of Comparative Physiology B 180:301-311.
Reinhold, K. 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. Functional Ecology 13:217-224.
Rezende, E. L. 2013. Better oxygen delivery. science 340:1293-1294.
Rezende, E. L., F. Bozinovic, and T. G. Jr. 2004. Climatic adaptation and the evolution of basal and maximum rates of metabolism in rodents. Evolution 58:1361-1374.
Rezende, E. L., and J. A. F. Diniz-Filho. 2012. Phylogenetic analyses: comparing species to infer adaptations and physiological mechanisms. Comprehensive Physiology 2:639-674.

Rezende, E. L., D. L. Swanson, F. F. Novoa, and F. Bozinovic. 2002. Passerines versus nonpasserines: so far, no statistical differences in the scaling of avian energetics. Journal of Experimental Biology 205:101-107.
Rolfe, D. F., and G. C. Brown. 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiological Reviews 77:731-758.
Rubenstein, D. R. 2012. Family feuds: social competition and sexual conflict in complex societies. Philosophical Transactions of the Royal Society B: Biological Sciences 367:2304-2313.
Rummer, J. L., D. J. McKenzie, A. Innocenti, C. T. Supuran, and C. J. Brauner. 2013. Root effect hemoglobin may have evolved to enhance general tissue oxygen delivery. Science 340:1327-1329.
Sadowska, E. T., M. K. Labocha, K. Baliga, A. Stanisz, A. K. Wróblewska, W. Jagusiak, and P. Koteja. 2005. Genetic correlations between basal and maximum metabolic rates in a wild rodent: consequences for evolution of endothermy. Evolution 59:672-681.
Schmidt-Nielsen, K. 1972. Locomotion: energy cost of swimming, flying, and running. Science 177:222-228.
Schmidt-Nielsen, K. 1984, Scaling: why is animal size so important?, Cambridge University Press.
Stearley, R., and G. Smith. 1993. Phylogeny of the Pacific trouts and salmons (Oncorhynchus) and genera of the family Salmonidae. Transactions of the American Fisheries Society 122:1-33.
Stoffels, R. J. 2015. Physiological Trade-Offs Along a Fast-Slow Lifestyle Continuum in Fishes: What Do They Tell Us about Resistance and Resilience to Hypoxia? PloS one 10:e0130303.
Swanson, D. L., N. E. Thomas, E. T. Liknes, and S. J. Cooper. 2012. Intraspecific correlations of basal and maximal metabolic rates in birds and the aerobic capacity model for the evolution of endothermy. PLoS ONE 7:e34271.
Teletchea, F., V. Laudet, and C. Hänni. 2006. Phylogeny of the Gadidae (sensu Svetovidov, 1948) based on their morphology and two mitochondrial genes. Molecular phylogenetics and evolution 38:189-199.
Teng, H.-Y., Y.-S. Lin, and C.-S. Tzeng. 2009. A new Anguilla species and a reanalysis of the phylogeny of freshwater eels. Zoological Studies 48:808-822.
Thompson, G. G., and P. C. Withers. 1997. Standard and maximal metabolic rates of goannas (Squamata: Varanidae). Physiological Zoology 70:307-323.
Tsuboi, M., A. Husby, A. Kotrschal, A. Hayward, S. D. Buechel, J. Zidar, H. Løvlie et al. 2015. Comparative support for the expensive tissue hypothesis: Big brains are correlated with smaller gut and greater parental investment in Lake Tanganyika cichlids. Evolution 69:190-200.
Watanabe, Y. Y., K. J. Goldman, J. E. Caselle, D. D. Chapman, and Y. P. Papastamatiou. 2015. Comparative analyses of animal-tracking data reveal ecological significance of endothermy in fishes. Proceedings of the National Academy of Sciences 112:6104-6109.
Wegner, N. C., O. E. Snodgrass, H. Dewar, and J. R. Hyde. 2015. Whole-body endothermy in a mesopelagic fish, the opah, Lampris guttatus. Science 348:786-789.
Welsh, J. Q., and D. R. Bellwood. 2012. Regional endothermy in a coral reef fish. PloS one 7:e33187.
White, C., and M. Kearney. 2013. Determinants of inter-specific variation in basal metabolic rate. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 183:1-26.
White, C. R., and R. S. Seymour. 2004. Does basal metabolic rate contain a useful signal? Mammalian BMR allometry and correlations with a selection of physiological, ecological, and life-History variables. Physiological and Biochemical Zoology 77:929-941.
Wiersma, P., M. A. Chappell, and J. B. Williams. 2007. Cold- and exercise-induced peak metabolic rates in tropical birds. Proceedings of the National Academy of Sciences 104:20866-20871.
Wone, B., M. W. Sears, M. K. Labocha, E. R. Donovan, and J. P. Hayes. 2009. Genetic variances and covariances of aerobic metabolic rates in laboratory mice. Proceedings of the Royal Society of London B: Biological Sciences 276:3695-3704.

Zhang, Y., Q. Huang, S. Liu, D. He, G. Wei, and Y. Luo. 2014. Intraspecific mass scaling of metabolic rates in grass carp (Ctenopharyngodon idellus). Journal of Comparative Physiology B 184:347-354.

Table 1. Comparison of PGSL models investigating how resting metabolic rate (RMR) and maximum metabolic rate (MMR) are related to species ecology across diverse teleost species. λ = phylogenetic correlation ranging between 0 and 1 (star and original phylogeny, respectively), AIC = Akaike's information criterion, w_i = Akaike's weight.

Variable	Model	λ	AIC	w_i
RMR	standard (body mass + temperature)	0.531	48.23	0.30
(n = 108)	standard + trophic level	0.526	49.99	0.12
	standard + lifestyle	0.396	47.54	0.42
	standard + lifestyle + trophic level	0.394	49.47	0.16
MMR	standard (body mass + temperature)	0.632	13.47	0.01
(n = 77)	standard + trophic level	0.586	13.00	0.01
	standard + lifestyle	0.472	6.47	0.37
	standard + lifestyle + trophic level	0.386	5.49	0.60

Table 2. Relationships of resting metabolic rate (RMR) and maximum metabolic rate (MMR), adjusted to body mass and temperature, with various morphological traits, adjusted for body mass.

	RMR					MMR				
	n	estimate ± SE	P	r^2	λ	n	estimate ± SE	P	r^2	λ
brain mass (%)	26	0.050 ± 0.296	0.868	0.001	0.55	23	-0.188 ± 0.317	0.559	0.02	0.79
hepatosomatic index (%)	25	0.067 ± 0.035	0.068	0.13	0.74	16	0.024 ± 0.066	0.725	0.01	0.98
gill surface area (log_{10})	30	0.142 ± 0.138	0.313	0.03	0.56	26	0.382 ± 0.158	0.023	0.24	0.74 [a]
muscle protein (%)	51	0.045 ± 0.019	0.026	0.09	0.60	44	0.083 ± 0.017	<0.001	0.35	0.32
caudal fin aspect ratio	26	0.081 ± 0.028	0.007	0.25	0.64	23	0.099 ± 0.024	<0.001	0.43	0.98

[a] This relation is obtained following the removal of *Sander lucioperca* (Cook's D = 0.621). Including this species, the relation is 0.186 ± 0.143 (P = 0.206, r^2 = 0.06, λ = 0.85).

Figure captions

Figure 1. Phylogeny, ecology and energetics of teleost fish. Phylogenetic hypothesis for the species in this study, with branch lengths in millions of years. The root node was set to 300 mya based on the estimated divergence between Elopomorpha and other teleosts (Near et al. 2012). Species were classified according to their lifestyle as benthic (B), benthopelagic (BP) and pelagic (P). Resting (RMR) and maximum metabolic rates (MMR) are adjusted for body mass and temperature, and shown for a standard fish of 1 kg measured at 15 °C.

Figure 2. Relationship between adjusted resting (RMR) and maximum metabolic rate (MM; panel A). Note that pelagic species tend to exhibit higher RMR and MMR than their counterparts with a benthic or benthopelagic lifestyle (Table 2). Adaptation for increased active metabolic rate (MMR) across species of teleost fish is associated with an increased resting metabolic rate (RMR; panel B). However, this increase in maintenance cost is less than proportional to the increase in MMR, and thus investment in MMR can lead to a substantial increase in (absolute) aerobic scope (AS, the difference between RMR and MMR; vertical lines in panel B and the solid line in panel C). In agreement with the aerobic capacity model, factorial aerobic scope (FAS) is relatively constant with changes in MMR due to correlated changes in RMR (panel D). All metabolic rate values shown are standardised for mass (1kg), temperature (15°C) and phylogeny.

Figure 3. Interspecific variation of teleost metabolism in relation to various morphological traits. Adjusted estimates of resting (RMR) and maximum metabolic rates (MMR) plotted against (A) muscle protein content, (B) caudal fin aspect ratio and (C) mass-adjusted gill surface area. Lines of best fit derived from PGLS analyses are included where we detected statistical evidence for a relationship (Table 3). For gill surface area, the line was obtained

after removing the influential point (Cook's D = 0.621) highlighted by the arrow (Table 3). As shown in (D), (E) and (F), these subordinate traits were positively correlated and were consistently higher in pelagic species after accounting for phylogenetic effects (see main text).

Figure 4. Continuum of ecological pressures, lifestyles, and resultant physiological traits across species of teleost fish. Shown are exemplar species across this continuum. A species at one end of the continuum will not necessarily have all traits, or be exposed to all pressures, listed. Images provided by Kim Kraeer, Lucy Van Essen-Fishman (*Pagrus auratus*) and Tracey Saxby (*Thunnus albacares*), Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/).

Figure A1. Mass-scaling and temperature effects on metabolic rates of teleosts. (A) Adjusted estimates of resting (RMR) and maximum metabolic rates (MMR), calculated for a standard temperature of 15ºC, plotted against body size, and (B) adjusted RMR and MMR calculated for a standard body mass of 1 kg plotted against temperature. Best fit regression lines derived from PGLS analysis are presented (for the full model, see main text).

Figure A2. Frequency distributions for resting metabolic rate (RMR; upper panel) and maximum metabolic rate (MMR; lower panel) standardised for mass (1 kg) and temperature (15°C), for all fish species included in analyses. Bin widths are 10 and 50 for the upper and lower panels, respectively, and in each case counts are number of species. RMRs range between 9.3 and 347.8 g O_2 h^{-1}, representing a 38-fold span, while MMRs range between 59.9 and 1724.8 g O_2 h^{-1}, representing a 29-fold span. Even when disregarding the relatively few species with exceptionally high metabolic rates, often but not always represented by pelagic species and illustrated by lighter-coloured bars, the range in RMR and MMR still represents a 24-fold and 14-fold difference, respectively.

Figure 1

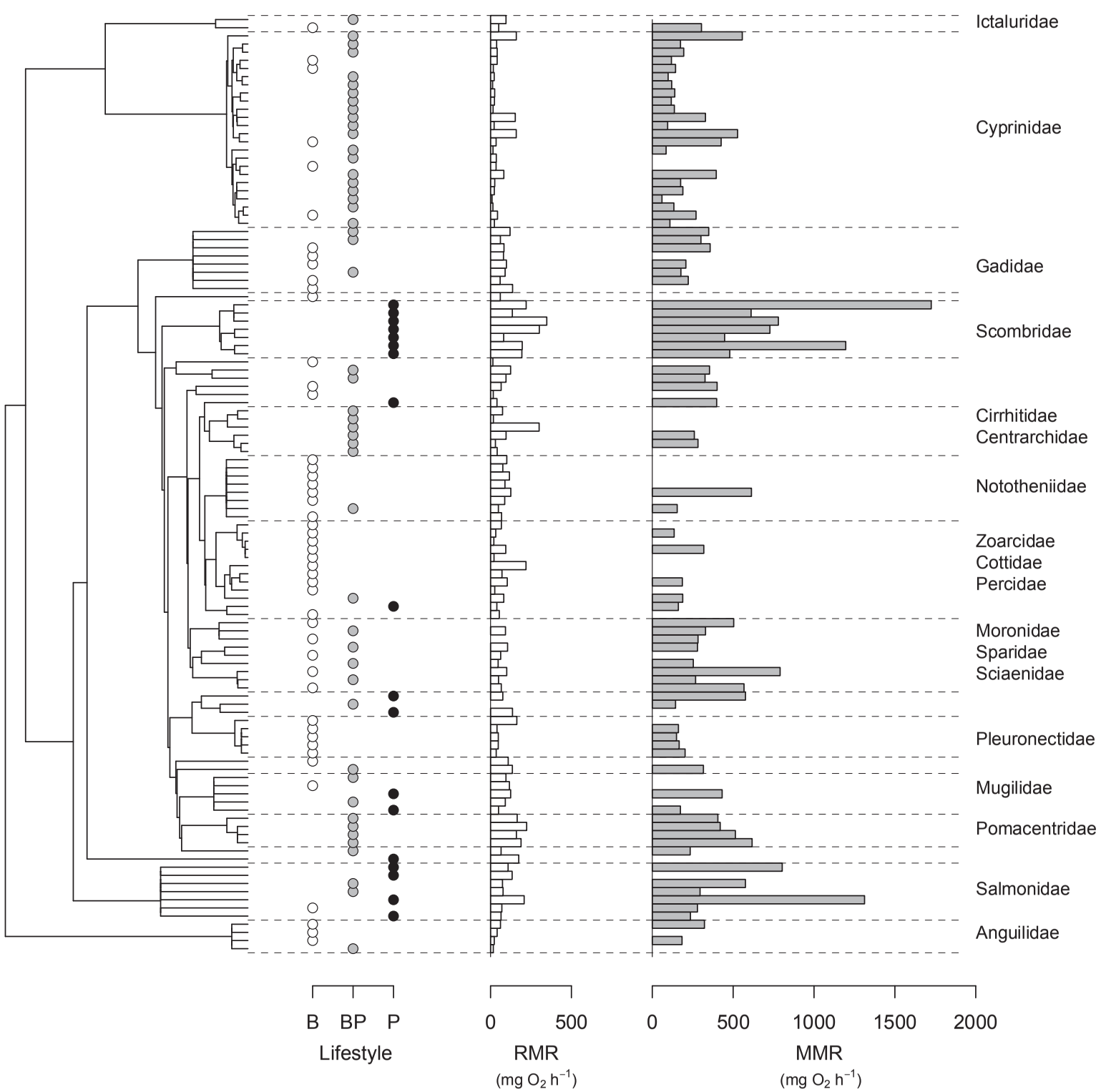
Ictaluridae
Cyprinidae
Gadidae
Scombridae
Cirrhitidae
Centrarchidae
Nototheniidae
Zoarcidae
Cottidae
Percidae
Moronidae
Sparidae
Sciaenidae
Pleuronectidae
Mugilidae
Pomacentridae
Salmonidae
Anguilidae
B BP P
Lifestyle
0 500
RMR
(mg O2 h−1)
0 500 1000 1500 2000
MMR
(mg O2 h−1)

Figure 2

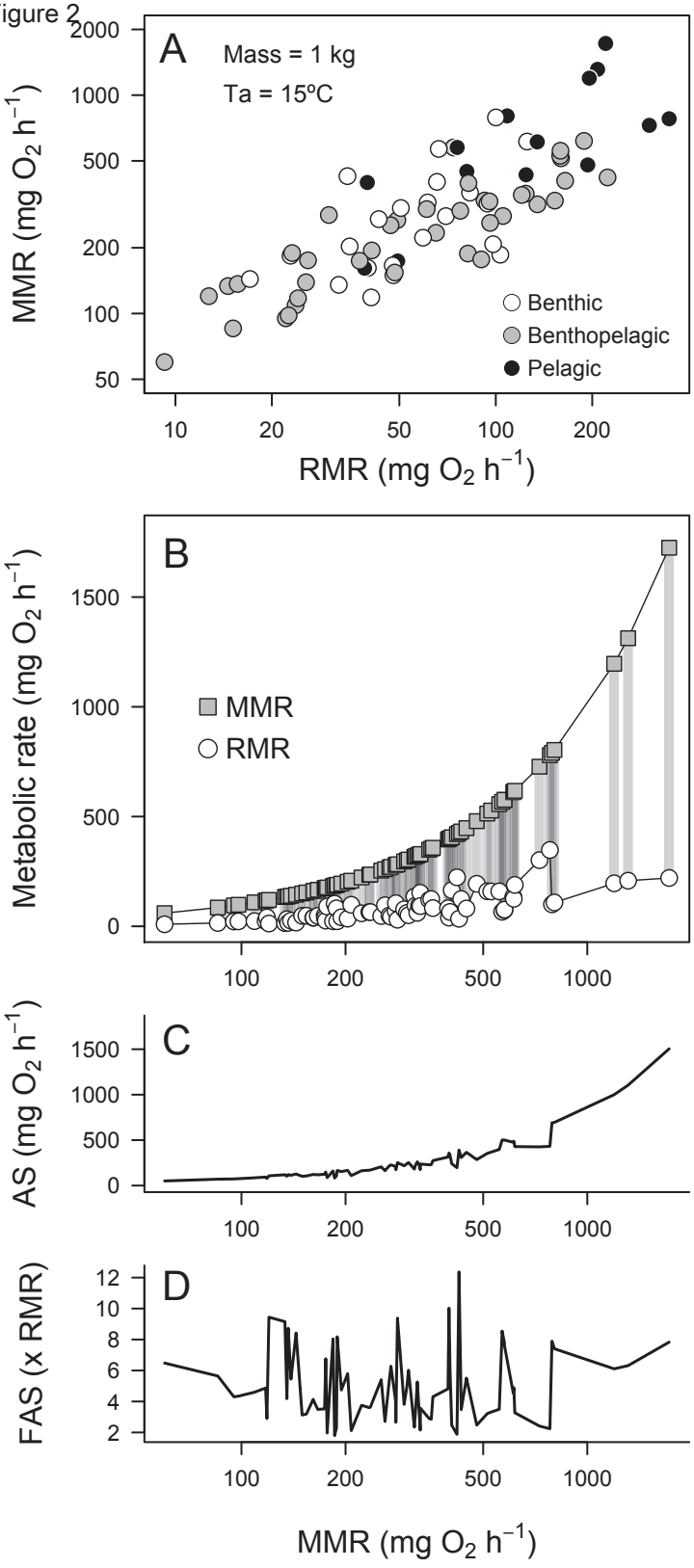
A
Mass = 1 kg
Ta = 15ºC
MMR (mg O2 h−1)
RMR (mg O2 h−1)
Benthic
Benthopelagic
Pelagic
B
Metabolic rate (mg O2 h−1)
MMR
RMR
C
AS (mg O2 h−1)
D
FAS (x RMR)
MMR (mg O2 h−1)

Figure 3

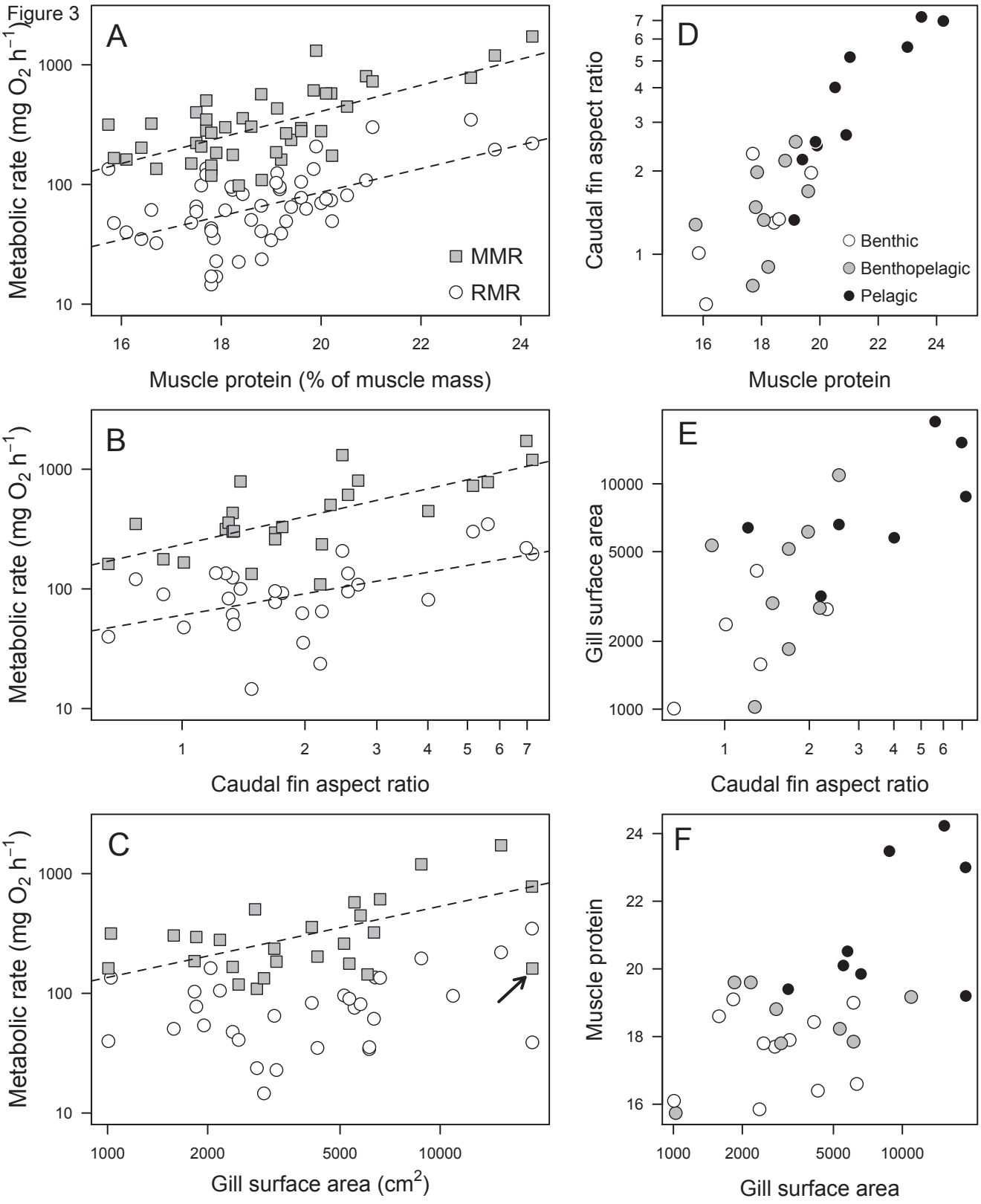
A
Metabolic rate (mg O_2 h^{-1})
1000
100
10
16
18
20
22
24
Muscle protein (% of muscle mass)
MMR
RMR
D
Caudal fin aspect ratio
7
6
5
4
3
2
1
Benthic
Benthopelagic
Pelagic
Muscle protein
B
Metabolic rate (mg O_2 h^{-1})
1000
100
10
1
2
3
4
5
6
7
Caudal fin aspect ratio
E
Gill surface area
10000
5000
2000
1000
1
2
3
4
5
6
Caudal fin aspect ratio
C
Metabolic rate (mg O_2 h^{-1})
1000
100
10
1000
2000
5000
10000
Gill surface area (cm^2)
F
Muscle protein
24
22
20
18
16
1000
2000
5000
10000
Gill surface area

Figure 4

Carpio carpio		*Thunnus albacares*
low oxygen or food availability low locomotory demands	**Ecological Pressures**	high oxygen or food availability high locomotory demands
benthic sluggish sit-and-wait/slow forager	**Lifestyle**	pelagic athletic active forager
low RMR/MMR/AS	**Physiology**	high RMR/MMR/AS high muscle protein large gill surface area

Figure A1

Supplementary Figure

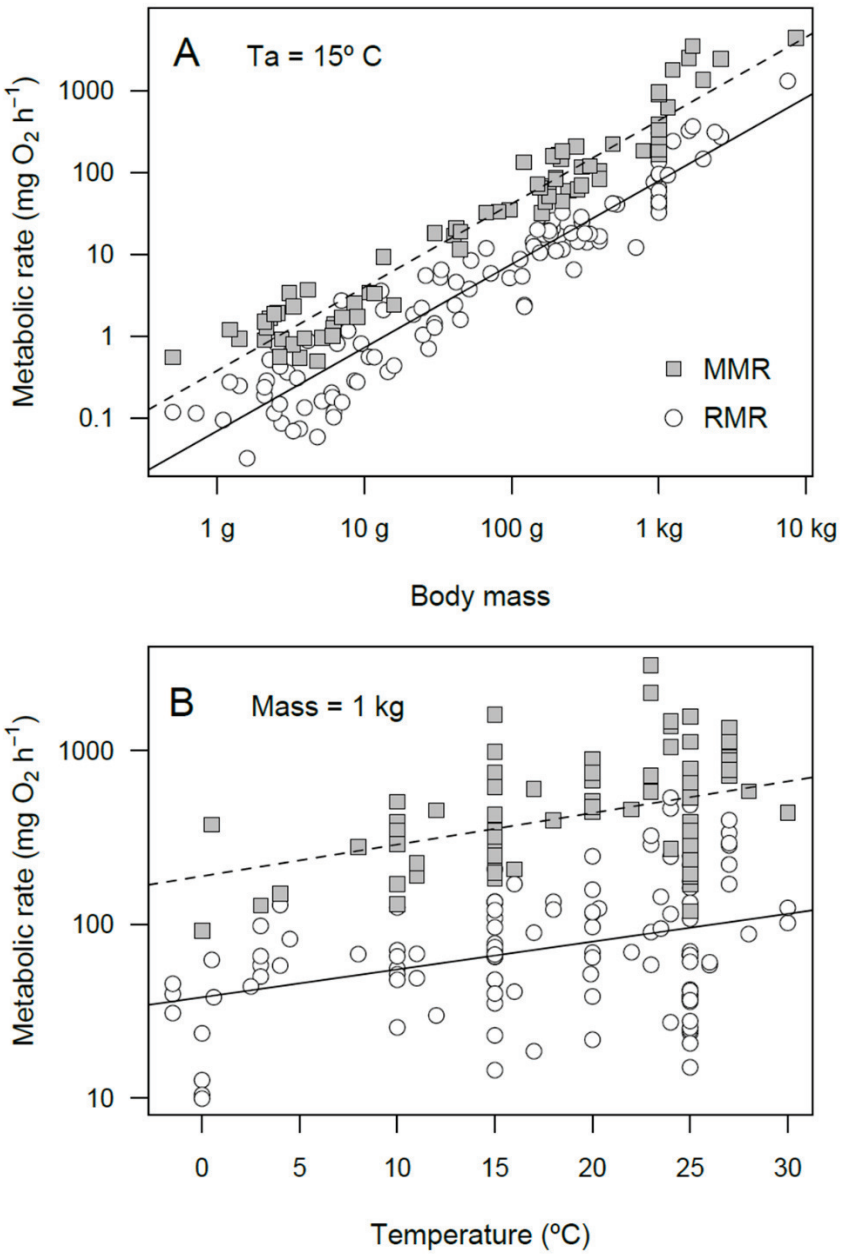

Figure A2

Supplementary Figure

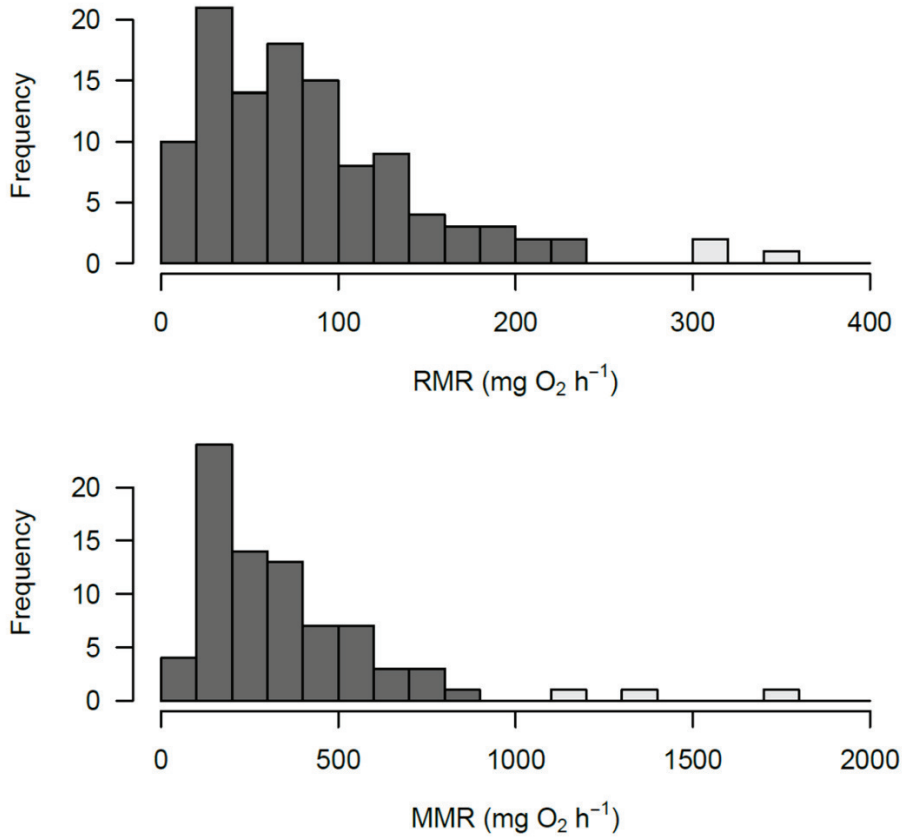

Table S1

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Other (Video, Excel, large data files)

Killen et al Am Nat Table S1.xlsx